# AIR-DRY METHOD FOR STUDYING CHROMOSOMES OF INSECTS AND ARACHNIDS[1,2]

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ABSTRACT: A rapid air-drying technique with giemsa staining is described for use on insects and arachnids. Time-saving steps have been made to result in a relatively fast, easy method which can be performed in the field. Both meiotic and mitotic cells can be successfully treated with colchicine to increase the number of metaphase cells, although some polyploid nuclei are sometimes produced.

We present a simple and rapid air-drying technique for preparing chromosomes of different arthropods for karyotypic analysis. It has been used sucessfully with ants, beetles, moths, spiders, harvestmen, and scorpions. Advantages of the present method are numerous and lead to a relatively fast, simple method suitable for field use: (1) numerous specimens of a single population or species can be simultaneously prepared up to the point of cell dissociation, (2) nicely spaced cell preparations are obtained without the use of enzymes, centrifuging, or resuspending of cells, (3) cover slips, dry ice, or liquid nitrogen are not needed, (4) preparations when protected from dust and held at room temperature are relatively stable for at least one year, (5) preparations prior to staining can be treated for banding patterns.

## MATERIALS

Equipment:

- Dissection microscope (optional, depending on tissue studied)
- Watch glasses or depression slides
- Disposable pipets
- Stainless steel rod: smooth, flat bottomed, 5-7 mm diameter
- Microscope slides: pre-cleaned in acid-alcohol (10 ml conc. HCl, 1000 ml 70% ethanol in $H_2O$), rinsed in two changes of absolute ethanol, flame dried
- Fine-tipped forceps
- Iris scissors, microscalpels, teasing pins (optional)

Solutions:

- Ringer's solution: 14.0 gm NaCl, 0.2 gm KCl, 0.2 gm $NaHCO_3$, 0.4 gm $CaCl_2$, 1000

[1]Received August 29, 1984. Accepted December 10, 1984.

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ml distilled $H_2O$

Colchicine solutions: 0.01% or 0.005% w/v colchicine, Ringer's solution. Keep frozen and protected from light until ready to use, or on ice for a few days in the field. Alternatively, sterilized solutions can be prepared, which are stable for at least six months if protected from light (see Todd, 1967: 411).

Hypotonic solution: 5.6 gm KCl, 1000 ml distilled $H_2O$

Fixative: 1:3 glacial acetic acid, absolute methanol. Mix fresh batch for each series of preparations, making sure reagents are water-free (see Baker et al., 1982).

Dissociation solutions: (1) 6:1:1 glacial acetic acid, lactic acid, distilled $H_2O$; or (2) 60% v/v glacial acetic acid, distilled $H_2O$

Staining solution: 1:20 giemsa stain (Fisher Scientific SO-G-28 or equivalent), buffer

Buffer: 15 M Sorensen's pH 6.8 buffer, 4.54 gm $KH_2PO_4$ 4.75 gm $Na_2HPO_4$, 1000 ml distilled $H_2O$

Tissues:

As in other techniques, the tissue of choice is one in which numerous dividing cells can be found (Smith, 1943). Although other organs/tissues are suitable for study, we have concentrated on testes and ovaries of subadult (penultimate instar) and adult insects and arachnids, and cerebral ganglia of ant prepupae (last instar larvae ready to pupate).

## PROCEDURE

All steps below should be carried out at room temperature unless indicated otherwise.

1. Treat living specimen with colchicine solution, preferably at or near the animal's optimum temperature. The concentration and time of treatment is dependent on age and type of tissue being studied - see Results and Discussion.
2. Dissect desired tissue from specimen into Ringer's solution. Repeat the process if more tissue is desired.
3. Pipet off Ringer's solution, replacing with two rinses of hypotonic solution. If working with large samples, the tissue can be moved through the rinses by using fine-tipped forceps.
4. Treat with hypotonic solution for 15 minutes.
5. Rinse tissue twice (as in step 3) in freshly mixed fixative.
6. Treat with fixative solution for 15 minutes.
7. Place tissue (by use of forceps or dissecting needles, etc.) in one drop of dissociate solution on center of microscope slide, and rapidly smash with metal rod. The type of dissociate solution used depends on tissue - see Results and Discussion.
8. Immediately following dissociation (before the cell suspension dries or the dissociate solution destroys the preparation), two or three drops of freshly mixed fixative are used to spread the dissociated cells and to rinse the slide of dissociate solution. Cell spreading is aided by tilting slides back and forth.

9. Drain any excessive solutions from slides and allow to dry at a near vertical position for 24 hours.
10. Stain in freshly diluted giemsa stain for 9 minutes, then rinse slide by dipping two or three times in standing tap water and allow to dry.

## RESULTS AND DISCUSSION

Tissues treated with this method should result in preparations in which individual cells are dissociated, and spacing of chromosomes is such that they may be counted (Fig. 1).

Colchicine in the proper concentrations should produce an accumulation of meiotic and mitotic metaphase cells (c-metaphase of Levan in Kihlman, 1966). When working with adult arachnids (unless recently molted) treatment with colchicine is almost certainly required, whereas tissues from stages with active cell divisions may not require treatment, and in these situations colchicine should be avoided. Colchicine can cause problems in chromosome analysis: The arms of some chromosomes contract differentially (Smith, 1965) and polyploids can result (Fig. 2, 3). As the method of administering the colchicine also varies, some experimentation is needed. For ants, start with a 0.01% solution and follow methods of Crozier (1968). We have also found that prepupae and pupae can be treated by placing the punctured animal between sheets of Kimwipes® which are saturated with a colchicine solution. For spiders and other soft-bodied arachnids the entire animal can be dipped in a colchicine solution (start with 0.01%) and then allowed to drink the solution. Hard, large-bodied insects and arachnids can be injected with colchicine (start with 0.05 ml at 0.01% for medium-sized scorpion). Rocchi et al. (1984) report using a 0.05% solution for one hour on the isopod *Asellus aquaticus* (Linne). Colchicine does not affect all cells identically, so that concentrations and treatment times will vary. Some authors report no apparent effects of the drug on the cells (Cokendolpher and Francke, 1985; Mehlhop and Gardner, 1982). We have noted varying degrees of success in accumulating metaphase cells.

Under the "solutions" heading we list two different dissociation solutions. The one composed in part by lactic acid is very caustic to cells and often results in chromosomes that appear hazy. It is recommended when either hard or large masses of tissue are to be dissociated. Soft, small tissues are best treated in 60% acetic acid.

For field preparations of tissues from active subadult stages, treatment with colchicine can be omitted. Further, the slides can be stained upon returning to the laboratory. With these two steps deleted it takes only 30 minutes, plus time of dissection, to produce chromosome preparations. If the relative humidity in the field is high, slides will not dry rapidly. To aid

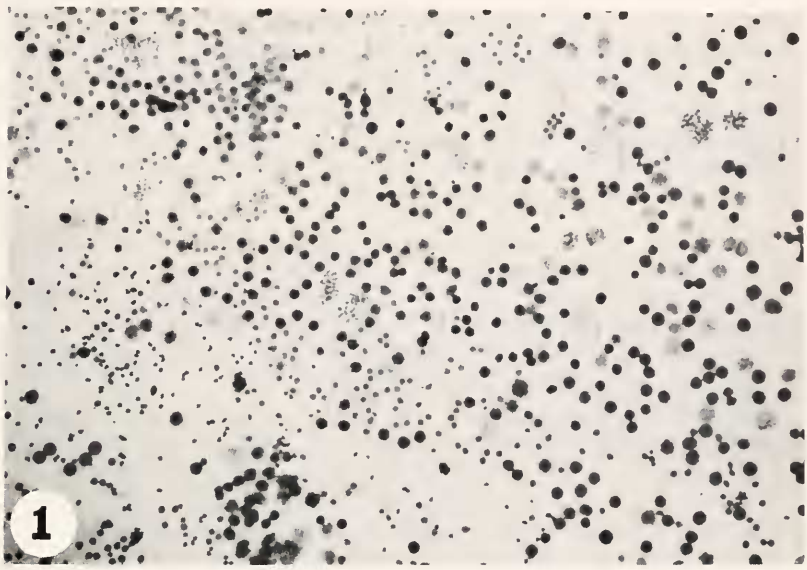


Fig. 1. Chromosome preparation from testis of the pholcid spider *Physocyclus* sp: cell spacing after 60% acetic acid dissociation.

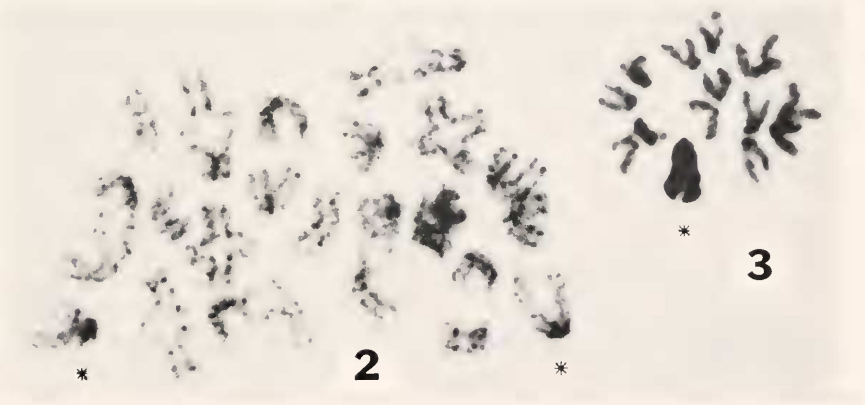


Fig. 2, 3. Chromosome preparations from testis of the pholcid spider *Physocyclus* sp. 2, karyotype of polyploid (diakinesis), tissue treated 24 hours with 0.005% colchicine. 3, karyotype (metaphase, 2n=15), without colchicine treatment. Asterisks indicate X chromosomes.

drying, ignite the fixative at the end of step 8 (see Mehlhop and Gardner, 1982).

We have not experimented with banding to any extent, but some spider cells in metaphase show faint banding patterns without further treatment. Those desiring to attempt chromosome banding should consult Steiniger and Mukherjee (1975). The present procedure can be modified to follow theirs by altering the fixative and fixing time: 3:1 glacial acetic acid, absolute methanol for 5-8 minutes.

## ACKNOWLEDGMENTS

Nobuo Tsurusaki of Hokkaido University and Jorge Santiago-Blay of University of Puerto Rico are sincerely thanked for their advice and discussions on chromosome preparation methods. Oscar F. Francke, Frederick B. Stangl, Jr., Stephen W. Taber (all of Texas Tech University), and Norman V. Horner (Midwestern State University) kindly reviewed the manuscript.

The senior author was supported by the Texas Department of Agriculture Interagency Agreement IAC (83-84)-0853.

## LITERATURE CITED

Baker, R.J., M.W. Haiduk, L.W. Robbins, A. Cadena, and B.F. Koop. 1982. Chromosomal studies of South American bats and their systematic implications. Spec. Publ. Pymatuning Lab. Ecol., No. 6, pp. 303-327.

Cokendolpher, J.C. and O.F. Francke. 1985. Karyotype of *Conomyrma flava* (McCook) (Hymenoptera: Formicidae). J. New York Entomol. Soc., 92(4): 349-351.

Crozier, R.H. 1968. An acetic acid dissociation, air-drying technique for insect chromosomes, with aceto-lactic orcein staining. Stain Technol., 43: 171-173.

Kihlman, B.A. 1966. Actions of chemicals on dividing cells. Prentice-Hall, Inc.: Englewood Cliffs, New Jersey, 259 pp.

Mehlop, P. and A.L. Gardner. 1982. A rapid field technique for preparing ant chromosomes for karyotypic analysis. Stain Technol., 57: 99-101.

Rocchi, A., G. Prantera, V. Lanza, and M. Di Castro. 1984. Incipient sex chromosome differentiation in an isopod crustacean species, *Asellus aquaticus*. Chromosoma, 89: 193-196.

Smith, S.G. 1943. Techniques for the study of insect chromosomes. Canadian Entomol., 75: 21-34.

Smith, S.G. 1965. Heterochromatin, colchicine, and karyotype. Chromosoma, 16: 162-165.

Steiniger, G.E. and A.B. Mukherjee, 1975. Insect chromosome banding: Technique for G- and Q-banding pattern in the mosquito *Aedes albopictus*. Canadian J. Genet. Cytol., 17: 241-244.

Todd, R.G. (ed.) 1967. Extra Pharmacopoeia. The Pharmaceutical Press: London, 25th ed., xxviii + 1804 pp.